REMARKS/ARGUMENTS

The present amendment is submitted in accordance with the Revised Amendment Format as set forth in the Notice provided on the USPTO web site for the Office of Patent Legal Administration; Pre-OG Notices; signed 1/31/03.

Claims 1-6, 8-13, 15 and 17-30 are pending. Claims 1-3, 19, 21 and 26 would be canceled without prejudice. Claims 31-33 are newly presented. Claims 4, 8, 11, 15, 17, 20, and 27-30 would be amended. After entry of these amendments claims 4-6, 8-13, 15, 17, 18, 20, 22-25, and 27-33 would be pending.

Status of the claims.

The subject matter of claims 11-13 and 1-6, 8-10 and 17-30 stands rejected allegedly obvious over Della Valle, et al. (1997) in view of Cooper, et al. (1993), Hansbrough, et al. (1989) and Myers, et al. (1997). Applicants respond to this rejection below.

Status of the Specification.

The previously submitted amendments to the specification (A-D, F, G and J) were rejected for the alleged entry of new matter. Applicants cancel those amendments and present new amendments A-D, F, G and J to address the Examiner's concerns. Applicants thank the Examiner for taking time to discuss the proposed substitute amendments.

Amendments to the Claims.

Claim 4 would be amended to recite "A method for cultivating a skin material for grafting onto a neodermis of a human patient." Support for this subject matter is found, *inter alia,* in the first sentence of the Summary at p. 4, the previous version of the claims and original claims 15-17, as well as the Title of the application.

Claims 8 and 11 would be amended to recite "a skin material for grafting onto a neodermis of a human patient." Support for this subject matter is set forth as above.

Claim 15 would be amended to recite a "A method for grafting a cultivated skin material onto a human patient." Support for the "cultivated" subject matter is found throughout the specification and, *inter alia*, in original claims 1-10.

Claims 17 and 20 would be amended to correct their dependencies in view of canceled intervening claims. Support for the subject matter of these claims is found, *inter alia,* in the previous versions of the claims.

Claims 27-30 would be amended to recite "wherein the membrane has holes capable of draining exudate." Support for this subject matter is found in the specification in the legend of Figure 1A and the figure itself which shows such holes.

Claim 31 is new. Support for the subject matter of claim 31 is found, *inter alia,* in original claim 15 and in the Abstract of the specification as originally filed.

Claim 32 depends from claim 31 and recites "wherein said keratinocytes are autologous to the keratinocytes." Support for this subject matter is found, *inter alia,* in original claims 10 and 13.

Claim 33 depends from claim 31 and recites "wherein said cultivated skin material further comprises a layer of dermal fibroblasts upon said basal side of said biosynthetic substratum." Support for this subject matter is found, *inter alia,* in original claims 11 and 17.

In view of the above, Applicants believe the amendments to the claims add no new matter and respectfully request their entry.

Amendments to the Specification.

Amendments A-D, F, G and J recite the phrase "LASERSKIN^{TM} artificial skin" in place of "Laskerskin" and/or also recite the phrase "INTEGRA^{TM} artificial skin" in place of "Integra^{TM}."

In view of the above, Applicants believe the amendments to the specification present no new matter and respectfully request their entry.

Response to Rejections under 35 U.S.C. §103(a).

The subject matter of claims 11-13 and 1-6, 8-10, and17-30 is alleged to be obvious over Della Valle, et al. (1997) in view of Cooper, et al. (1993), Hansbrough, et al. (1989) and Myers, et al. (1997). The reasons set forth by the Examiner are presented on pages 7-9 of Paper 4, pages 8-9 of Paper No. 7, and pages 5 and 6 of Paper No. 13, the final Office Action. Applicants respectfully disagree and respond to the remaining 35 U.S.C. §103 rejections below.

Without acquiescing to the position of the Examiner and in order to expedite prosecution of the application, Applicants have canceled some claims and amended base claims 4, 8, 11 and 15 as provided above.

Standard of Review.

As set forth in M.P.E.P. § 2143:

> [t]o establish a *prima facie* case of obviousness, *three* basic criteria must be met. *First*, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. *Second*, there must be a reasonable expectation of success. *Finally*, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)."

The *Prima Facie* Case of Obviousness

For the convenience of the Examiner, Applicants reproduce below the fundamental basis of these rejections as set forth on pages 7-9 of Paper 4:

> It is well known in the art that the presence of a dermal layer consisting in part of fibroblasts contributes to improved graft "take rates." For example, Cooper et al.

write: "In skin substitutes, epidermal growth has been increased with the addition of viable fibroblasts … human keratinocytes alone on collagen glycosaminoglycan had poor take on athymic mice, and that the addition of human fibroblasts more than doubled the acceptance and persistence of the grafts on mice (see p.187, 1st column). In the experiments of Cooper et al. they compare the engraftment of composite skin consisting of keratinocytes and fibroblasts to that of epidermal sheet grafts consisting of cultured keratinocytes alone. For the composite skin, fibroblasts were seeded in a collagen matrix and later keratinocytes seeded on a non-porous side of the composite. Cooper et al. teaches that the inclusion of fibroblasts in graftable material increases the production of collagen in vitro, results in the production of laminin at the appropriate dermoepidermal junction in vivo and improves the quality of the dermoepidermal junction compared to keratinocyte sheet grafts. Cooper et al. conclude: … addition of fibroblasts in the graft along with keratinocytes enhances formation of basement membrane proteins, which improves attachment of the epidermal layer." (see page 817-818). The results presented in this paper are an extension of work published in Hansbrough et al. in which collagen aminoglycan membranes were used to autograft cultured fibroblasts and keratinocytes in burn patients.

Meyers et al. teaches the use of a hyaluronic acid membrane for the delivery of cultured keratinocytes. The method involves the culturing of autologous keratinocytes on a perforated hyaluronic membrane in the presence of irradiated non-proliferating fibroblast cells. Experiments described in Meyers et al. include the comparison of grafting in the presence and absence of a dermal layer. Dermal layers in would areas where generated by autografting de-epidermalised dermis (which consists essentially of fibroblasts). The data of Meyers' indicates that the best results were achieved with keratinocytes cultured on hyaluronic acid membranes and grafted onto wounds which contained a dermal layer (i.e., which had fibroblasts present. (see materials and methods section , pages 215-218).

It would be readily apparent to one of skill in the art that a reasonable application of hyaluronic membranes for grafting would incorporate the teachings of Cooper et al. - indicating that the co-culturing of live fibroblasts with keratinocytes in cultivated skin material provides advantages over keratinocytes alone, - and the teachings of Meyers et al. which points to the need of fibroblast (dermal) cells for better graft formation. Both point to a crucial interaction of fibroblasts and keratinocytes in

> graft formation. Hansbrough et al. teach the use of autologous fibroblasts and keratinocytes in a collagen-glyscosaminoglycan matrix anticipating the use of biosynthetic substratum containing autologous and allogenic cells (see page 2125, column 3- Materials and Methods). Given the directionality of the dermo-epidermal junction, it would be prima facie obvious to culture keratinocytes and fibroblasts as disclosed in claim 4 with one side of the membrane facing the developing dermis and consisting of fibroblasts and a second side of keratinocytes underlaid with fibroblasts. The culturing of keratinocytes over viable fibroblasts on one face of the membrane is an obvious extension of the use of 3T3 feeder cells and further anticipates the need for a good epidermal junction.

Disclosures of the Cited References.

Applicants next address the salient features of what the cited references disclose.

1. The Della Valle, et al. reference.

The Action interprets the Della Valle, et al. reference as teaching a product 'epidermal' artificial skin which consists of murine 3T3 fibroblasts and human keratinocytes. In support of this position, the Action cites col. 5, lines 3-14, of the Della Valle, et al. reference. This section recites "After adhesion of the 3T3 cells, i.e., after 24 hours, ...". Applicants respectfully disagree with the above interpretation of "after adhesion" for the following reasons.

Applicants submit with the IDS the Green, et al. reference (Green, et al., *PNAS*, 76:11, pp. 5665-5668 (1979)), which is cited by Della Valle, et al. at col. 4, lines 43-45 of Example 2 as the source of their method. The sections of Example 3, relied upon by the Examiner (at col. 5, line 5), state the subject material was cultivated with the conditions of Example 2. Green, et al. teach at p. 5666, first column, first full paragraph:

> "Keratinocyte colonies appears within a few days and, as they expanded, they displaced the 3T3 cells in typical fashion."

Thus, one of ordinary skill in the art would know that the 3T3 cells upon incubation with the keratinocytes become displaced and so could not become more than a contaminant of the Della Valle, et al. artificial epidermis.

Indeed, upon diligent review, Applicants could find no recital of the Della Valle, et al. reference which describes 3T3 feeder cells as an intended or actual component of a finished artificial skin. In all its descriptions of their artificial skin material, Della Valle, et al. do not appear to anywhere mention 3T3 cells as forming an integral part of their artificial skin material. For instance, the reference describes the artificial skin produced by the process of the above recital at col. 5, lines 31-42 thusly:

> The artificial skin according to the present invention, obtained by the aforesaid procedures, therefore consists of a biocompatible and preferably bioreabsorbable support membrane consisting of materials of natural, synthetic or semisynthetic origin, and having a thickness of between 10 and 500.mu., and preferably between 20 and 40.mu., characterised by comprising an ordered series of holes of a defined and constant size between 10 and 1000.mu., separated from each other by a constant distance of between 50 and 1000.mu., together with autologous or heterologous keratinocyte microcolonies in the active proliferation state present within the holes.

The above recital simply makes no mention of 3T3 feeder cells or fibroblasts. Nor, do Della Valle, et al. in describing their drawings make any mention of a fibroblast layer (see col. 2, lines 49-56). Rather, they state with respect to Figures 2-6 that they "show the growth of the keratinocytes layer on the membrane of hyaluronic acid benzyl ester." (underlining added for emphasis).

As reflected in its Title, Abstract and Claims, and throughout the specification, the Della Valle, et al. reference almost exclusively serves to disclose the laser drilled biosynthetic membranes for use in artificial skin. It recites, for instance, at col. 4, first two paragraphs:

> The perforated biocompatible membranes according to the present invention can be used advantageously for the in vitro culture of epithelial cells, especially keratinocytes.
>
> For this purpose the membranes can be fixed to the base of cell culture vessels, to metal grids or to any other structure suitable for cell cultures at the air/culture medium interface, using sterile vaselin, sterile silicone or other cementing systems which allow easy removal of the membrane, or by systems involving the use of

> biological material such as collagen, fibrin or fibrin glue. These membranes can be incubated in culture media suitable for the growth of epithelial cells either alone or in the presence of other cells, such as irradiated fibroblasts, as described in the cited literature, without within the time scheduled for growth and hole colonization causing alteration in mechanical properties which would compromise their handleability and strength within the particular application.

At col. 2, line 34, Della Valle, et al. state:

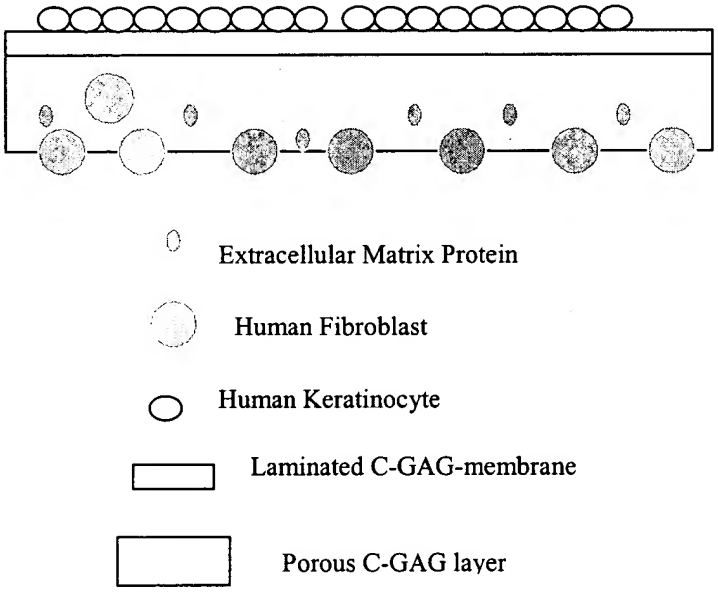
> A further object of the present invention is to provide a biocompatible and preferably bioreabsorbable artificial skin which can be produced in a short time, is strong, and is easily handled at the moment of transplantation, and which moreover can be applied to the site of the lesion independently of its original orientation in the culture vessel, and can be easily stored. In this respect, an advantage of the artificial skin according to the present invention is that it can be easily cryopreserved to allow the creation of a bank of epithelial tissue, including heterologous. The possibility of cryopreservation also considerably reduces or eliminates, after at least two cycles, *the antigenic potential of the surface antigens expressed by the epithelial cells*. (italics added for emphasis).

The recital focusing on the "antigenic potential of the surface antigens expressed by the epithelial cells" makes no sense if the murine 3T3 xenogenic cells are present in the material to be grafted.

Thus, Applicants submit that the 3T3 cells are simply not a structural element of the artificial skin material of Della Valle, et al. If they are present at all, it could be only as an unwanted minor contaminant and not a "layer."

2. The Action cited the Hansbrough, et al. (1989) and the Cooper, et al. (1993) references as disclosing a skin graft material having autologous keratinocytes and autologous fibroblasts. Applicants agree.

Both the Hansbrough, et al. and Cooper, et al. references disclose a composite of the following structure:



In this reference, autologous human fibroblasts and keratinocytes were each first cultured separately. The cultured fibroblasts were then seeded onto the porous surface of the membrane. Thereafter, the human keratinocytes are seeded onto the planar nonporous surface of a laminated collagen-glycosaminoglycan (C-GAG) membrane and allowed to proliferate (see Hansbrough, et al. at p. 2125, first two columns).

3. The Action cites Meyers, et al. as teaching the importance of a well-prepared wound bed, ideally, one which is live and autologous. Applicants agree.

Rebuttal of the prima facie case.

A. The Cited Art Does Not Suggest Making the Proposed Combination.

The cited references, taken alone or in combination, do not suggest or motivate their combination to provide the claimed compositions or methods. When considered for all that they teach, the cited references *teach away* from Applicants' claimed invention.

The Action relies upon Meyers, et al. for teaching the importance of a live and autologous wound bed (e.g., dermis). The Action alleges that one of ordinary skill would recognize that a live and autologous wound bed could be provided by a material "that has *keratinocytes* seeded on both sides of the biosynthetic substrate." (italics added for emphasis). As fibroblasts, and not keratinocytes, populate the dermal layer of normal skin, it appears most likely that the Examiner meant to allege that one of ordinary skill would recognize that a live and autologous wound bed could be provided by a material "that has *fibroblasts* seeded on both sides of the biosynthetic substrate." (However, if *keratinocytes* was the intended term, Applicants note that Meyers, et al. teach an advantage of a composition having *keratinocytes* seeded on both sides of a biosynthetic substrate at p. 220, first column and that the present claims are drawn in part to compositions and methods wherein only one side of the biosynthetic substrate of the artificial skin has the keratinocyte layer.)

Either way, the proposed solution is not a logical extension of the disclosures of Meyers, et al. or Della Valle, et al. who teach an advantage of an artificial skin product which can be inverted upon grafting. Meyers, et al. teach an advantage of having *keratinocytes* available to both sides of the biosynthetic stratum so as to avoid reorientation problems associated with inverting the graft material upon transplantation (see p. 220, first column, lines 3-14). Della Valle, et al. are in accord with respect to reorientation. They state at col. 2, lines 37-40, that an objective met by their synthetic skin is that it "can be applied to the site of the lesion independently of its original orientation in the culture vessel, ..." The Applicants' artificial skin has keratinocytes at the upper level and dermal fibroblasts beneath. Turning the artificial skin over, would require a large scale reorganization and reorientation of the graft material which would defeat the purpose of the claimed arrangement of artificial skin elements.

In addition, with respect to how to provide a prepared wound bed, Applicants' artificial skin material does not provide a prepared wound bed but is rather applied to a prepared wound bed. Indeed, claim 15 and its dependent claims are drawn to a method

wherein the Applicants' artificial material is applied to a wound bed of a vascularized biosynthetic neodermis.

With respect to claim 15, especially, Meyers, et al. point one of ordinary skill in a direction opposite to the claimed subject matter. Meyers, et al. only employ in their experiments prepared wound beds derived from dermis and containing live fibroblasts. Meyers, et al. describe cryopreserved allodermis as the "closest available alternative" to a *live* and autologous wound bed at p. 221, 2nd column, first paragraph. The neodermis of claim 15 comprises C-GAG which is a biopolymer which is not alive and not a cryopreserved allodermis.

B. ThereWas No Reasonable Expectation of Success for the Proposed Combination

The expectation of success must be found in the prior art, not the Applicants' disclosure.

A standard method for the cultivation of keratinocytes is the method of Green, et al. (Green, et al., *PNAS*, 76:11 (1979)). This method was used by Della Valle, et al. and Meyers, et al. Green, et al. teach the use of non-irradiated fibroblast cells in the culturing of keratinocytes as preferred over the use of viable fibroblasts which were reported to out compete keratinocytes and thus needed to be held in check (see Green, et al. at p. 5665, col., 2 section iii). Thus, the kind of substitution proposed by the Examiner is reported as unsuitable in the prior art. In view thereof, the expectation of success is not supported by the prior art.

C. The Cited Art when Combined Does Not Provide All the Limitations of the Claims

Base claims 4, 8, 11 and 15 are drawn in part to subject matter of an artificial skin material having a layer of viable human keratinocytes over a layer of dermal fibroblasts upon one side of a biosynthetic substratum. None of the cited references disclose a layer of keratinocytes over a layer of fibroblasts on one side of a biosynthetic substrate. As discussed above, the 3T3 fibroblasts of Della Valle, et al. simply do not persist when co-cultivated with keratinocytes on the biosynthetic substrate. None of the cited references

employ a keratinocyte layer over a fibroblast layer on the same side of a biosynthetic substratum. This consistently absent feature can not be supplied merely by deeming it an obvious one to be tried.

Claims 11 and dependent claims 17 and 20 are also drawn in part to subject matter of an artificial skin material with a layer of human fibroblasts on both sides of a biosynthetic membrane. None of the cited references have two layers of fibroblasts on each side of a biosynthetic membrane of an artificial skin material.

Claim 17 is drawn to the use of a cultivated skin material comprising a layer of fibroblasts upon a basal side of a biosynthetic substratum, a layer of keratinocytes overlying a layer of viable human dermal fibroblasts upon an upper side of the biosynthetic substratum wherein the material is applied to a vascularized neodermis of C-GAG. If the objective of providing a prepared wound bed which is living is suggested by Meyers, et al., this *particular* multistep recital of first providing a vascularized woundbed of neodermis of a C-GAG material and then later applying the skin graft materials as recited in the claims is not found or suggested in any combination of the references.

Typically, in contrast, others who have used fibroblasts in their dermis substitutes, have sought to provide a wound bed having a neodermis already seeded with fibroblasts. The Hansbrough, et al. reference cited by the Examiner, for instance, provides a composite skin material corresponding to both epidermal and dermal layers. The dermal layer is seeded with fibroblasts. Meyers, et al., for example, use debraded skin or de-epidermialized dermal allografts which contain fibroblasts. Harris, et al. (cited in the IDS) teach the use of a neodermis of either a biosynthetic substrate seeded with fibroblasts or a cadaveric allograft (later debraded to remove the epidermal layer) having fibroblasts.

In view of the above, Applicants respectfully request that the above rejections under 35 U.S.C. §103(b) be reconsidered and withdrawn.

If the Examiner believes a telephone conference would aid in the prosecution of this case in any way, please call the undersigned at 925-472-5000.

Respectfully submitted,

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